Doubled Haploidy Research for Saskatchewan Crops

Alison M.R. Ferrie

National Research Council – Plant Biotechnology Institute, 110 Gymnasium Place, Saskatoon, SK, S7N 0W9

Keywords: doubled haploid, embryogenesis, breeding, *Brassica*, Apiaceae

Abstract

Doubled haploidy methodology is commonly used in many agronomically important crops to speed the development of new cultivars. There are a number of advantages in using haploidy technology in both practical application (varietal development, mutagenesis, transformation) and basic research (genomics, biochemical, and physiological studies). Haploid plants are commonly produced using one of four methods: culture of anthers or microspores (androgenesis), culture of unfertilized ovules (gynogenesis), interspecific or intergeneric crosses followed by chromosome elimination, and by pollination with irradiated pollen. The most efficient method depends on the species. There are a number of factors affecting microspore embryogenesis including genotype, donor plant growth conditions, stage of microspore development, composition of the culture medium, and environmental conditions during culture. The frequency of embryo production will depend on whether or not these conditions are optimal and varies depending on the species. For the past 20 years, the National Research Council – Plant Biotechnology Institute has been developing doubled haploidy protocols in a number of different species. We have also been utilizing these protocols in basic and applied research.

Introduction

There is always a need to improve the crops that are grown in Saskatchewan by increasing yield, improving quality, and improving tolerance to diseases and pests. In general, it takes about 10 years to develop a new variety, although this depends on the species. The traditional plant breeding methods involve the time consuming process of self-fertilization or back-crossing in order to produce homozygous lines. Doubled haploid (DH) production is the quickest method for developing homozygous breeding lines. The production of doubled haploid lines from microspores or ovules makes it possible to obtain stable homozygous lines in a single step.

Development of doubled haploid plants

Haploid plants can be produced naturally but since the frequency is very low, this method is not beneficial for breeding programs. Haploids can be produced by *in vitro* methods such as culturing the male gametophyte (anthers or isolated microspores) or the female gametophyte (unfertilized ovules). Haploid plants have also been developed by interspecific or intergeneric crosses followed by chromosome elimination, and by pollination with irradiated pollen. The most efficient method depends on the species.

In many species the culture of isolated microspores or anthers is the most frequently used method of haploid production. This is not new technology but was first reported in the 1960's (Guha and Maheshwari, 1964). The early work was mostly with the Solanaceae species but has now been observed in over 250 species. With this method, every microspore is potentially capable of regenerating into a viable embryo and each plant would therefore represent the variation which exists in the population of microspores. Plant regeneration occurs either by direct embryogenesis or through callus formation followed by embryogenesis or organogenesis.

There are a number of factors affecting microspore embryogenesis including genotype, donor plant growth conditions, stage of microspore development, composition of the culture medium, and environmental conditions during culture (Ferrie and Caswell 2011). The frequency of embryo production will depend on whether or not these conditions are optimal. These embryos are similar to those found in seeds and will regenerate into plants. However, these plants are haploid (i.e. having half the chromosome number of a normal plant). Treatment with colchicine, a naturally occurring compound found in the autumn crocus, doubles the chromosome number creating a doubled haploid plant.

Application of doubled haploidy research

Doubled haploidy technology has been developed for a number of species. There are many advantages in using haploidy technology in practical application (varietal development, mutagenesis, transformation) and basic research (genomics, biochemical, and physiological studies) and this has been reviewed recently (Dunwell 2010, Ferrie and Möllers 2011).

Worldwide, over 200 DH cultivars have been reported from 12 species (Thomas et al. 2003). This list is now outdated and incomplete as some breeding organizations are retaining cultivar development information as proprietary. One advantage of using DH plant production in a crop improvement program is the shortening of the breeding cycle. Utilizing DH methodology in a breeding program can speed the development of new cultivars by 3 – 4 years (Ulrich et al. 1984). Other advantages include easier identification of recessive traits, more efficient selection, and being able to work with smaller population sizes. Furthermore, marker assisted breeding is more efficient in haploid embryos and/or plants as only a single allele is present at any given locus.

Microspores, since they are single cells and haploid, make ideal material for mutant selection. By the very nature of haploids, all recessive and dominant traits are readily expressed and are easily selectable in culture. One can make changes at the single cell level and then regenerate the cell to a plant. Traits can be fixed by chromosome doubling to achieve homozygosity. Another advantage of using microspores in mutagenesis is the large number of uniform cells that can be exposed to chemical or physical mutagens in a relatively small space and mutants can be selected by the appropriate selection pressure. There are examples in the literature of developing herbicide tolerant mutants (Swanson et al. 1988, 1989), disease resistant lines (Zhang and Takahata 1999) or alterations in the fatty acid profiles (Ferrie et al. 2008).

Similarly to microspore mutagenesis, the haploid system is well suited for genetic transformation and the newer methods of gene editing. This is due to ease of handling a large number of uniform cells or embryos, usually of high regenerative potential, and the ability to duplicate the introduced

trait during chromosome doubling for doubled haploid production. There are a number of techniques used for the introduction of foreign genes into plant cells, most of which have been used to some extent with microspores with varying degrees of success.

Doubled haploidy or microspore culture can be used for biochemical and physiological studies. The developmental sequence of microspore-derived haploid embryos closely approximates the zygotic counterpart and many of the biochemical pathways are similar. Uniform microspore-derived embryos can be easily obtained in large numbers from responding genotypes. Zygotic embryos can be used in such studies but must first be excised at the appropriate stage from the developing seed, which is very time consuming.

The *Brassica* microspore culture protocol is well established. At the Plant Biotechnology Institute, we have developed microspore mutagenesis protocols for *B. rapa, B. napus, B. juncea, B. nigra, B. oleracea,* and *B. carinata*. We are also developing novel microspore transformation methods in *B. napus, B. carinata,* and *B. juncea*. Over the years, we have worked in collaboration with breeding institutes in varietal development and with other groups in using doubled haploidy technology for biochemical, physiological, and genomic studies.

At the Plant Biotechnology Institute we are developing doubled haploidy protocols in a number of different species and utilizing these protocols in both practical application and basic research. Some examples include the Apiaceae family (e.g. dill, carrot, anise, caraway) in which there is breeding of these crops but very little doubled haploidy research. We have screened a number of Apiaceae species and have generated microspore-derived embryos and doubled haploid plants in 10 of 21 species evaluated (Ferrie et al. 2011b). Field trials of doubled haploid dill have shown that the DH lines generally are shorter than the parental cultivar, which is beneficial as lodging is a problem in dill. Some DH lines were higher yielding and some had higher oil levels (Ferrie et al. 2011a). These lines could be used in varietal development. We are currently developing doubled haploidy protocols for oat (*Avena sativa*) and *Camelina sativa* (Ferrie and Bethune 2011). In both cases, microspore-derived embryos and doubled haploid plants have been produced and experiments are underway to optimize the protocols.

Acknowledgements

The author acknowledges T. Bethune, K. Irmen, and M. Mykytyshyn for their technical expertise. Funding for the different projects was provided by the Saskatchewan Agriculture Development Fund and the Canola Council of Canada.

References

Dunwell JM (2010) Haploids in flowering plants: origins and exploitation. Plant Biotechnology Journal 8:377-424

Ferrie AMR, Bethune TD (2011) A microspore embryogenesis protocol for *Camelina sativa*, a multi-use crop. Plant Cell Tissue and Organ Culture 106:495-501

Ferrie AMR, Bethune T, Arganosa G, Waterer D (2011a) Field evaluation of doubled haploid plants in the Apiaceae: dill (*Anethum graveolens,* L.), caraway (*Carum carvi* L.), and fennel

(*Foeniculum vulgare* Mill.). Plant Cell Tissue and Organ Culture 104:407-413

Ferrie AMR, Bethune T, Mykytyshyn M (2011b) Microspore embryogenesis in the Apiaceae. Plant Cell Tissue and Organ Culture 104: 399-406

Ferrie AMR, Caswell KL (2011) Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production. Plant Cell Tissue and Organ Culture 104:301-309

Ferrie AMR, Möllers C (2011) Haploids and doubled haploids in *Brassica* spp. for genetic and genomic research. Plant Cell Tissue and Organ Culture 104:375-386

Ferrie AMR, Taylor DC, MacKenzie SL, Rakow G, Raney JP, Keller WA (2008) Microspore mutagenesis of *Brassica* species for fatty acid modifications: a preliminary evaluation. Plant Breeding 127:501-506

Guha S, Maheshwari SC (1964) *In vitro* production of embryos from anthers of *Datura.* Nature 204:497

Swanson E B, Coumans MP, Brown GL (1988) The characterization of herbicide tolerant plants in *Brassica napus* L. after *in vitro* selection of microspores and protoplasts. Plant Cell Reports 7:83-87

Swanson EB, Herrgesell MJ, Arnaldo M (1989) Microspore mutagenesis and selection: Canola plants with field tolerance to the imidazolinones. Theor Appl Genet 78:525-530

Thomas WTB, Forster BP, Gertsson B (2003) Doubled Haploids in Breeding. In: Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) Doubled Haploid Production in Crop Plants, A manual. Kluwer Academic Publishers, Dordrecht, pp 337-349

Ulrich A, Furtan WH, Downey RK (1984) Biotechnology and rapeseed breeding: some economic considerations. Science Council of Canada Report 67

Zhang F-L, Takahata Y (1999) Microspore mutagenesis and *in vitro* selection for resistance to soft rot disease in Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*). Breeding Science 49:161-166